



FBI Laboratory

DNA UNIT II

Mitochondrial DNA Sequencing Protocol

SUPERSEDED BY MtDNA Protocol

dated 6/99

5/98

EXHIBIT NO. C

all factors
5/99
6/99

Remove the mortars from the microcentrifuge and remove the remaining water with a pipette.

4. Place the grinders in a rack and place in a Stratalinker® (Stratagene®) for a minimum of 15 minutes.

3.1.2. DNA Extraction

1. Remove the hair to be extracted from the ethanol (or water) with clean forceps and examine it under a stereomicroscope for the possible presence of sheath material, surface debris, or bodily fluids.
2. Prepare a wash solution of 5% (w/v) Terg-a-zyme™ detergent.
3. Place an approximately 2 cm portion of the hair in a 1.5 mL plastic tube filled with approximately 1 mL of 5% Terg-a-zyme™ and place in a rack in an ultrasonic water bath. Agitate for approximately 20 minutes. Examine the hair for the presence of surface debris. If necessary, continue to wash the hair in the Terg-a-zyme™ solution until free of surface debris.
4. Briefly rinse the hair in 100% ethanol, followed by ddH₂O.
5. A reagent blank should be prepared for each grinder ~~to be~~ used. Prepare the reagent blank by placing 200 µL of **stain extraction buffer** (SEB) into the micro tissue grinder to be used. Briefly simulate grinding. Remove the pestle and transfer the liquid to a sterile 1.5 mL plastic tube. Set aside until step 16.
6. To the same grinder add 200 µL of stain extraction buffer. Place the hair fragment into the micro tissue grinder.
7. Move the pestle up and down to force the hair into the bottom of the mortar. Grind until fragments are no longer visible.
8. Remove the pestle from the mortar. If liquid is adhering to the pestle head, gently pass it along the inner lip of the mortar until liquid flows to the bottom of the mortar.
9. Transfer the homogenate liquid to a sterile 1.5 mL plastic tube.
10. Add 1 µL of 600 U/mL of **proteinase K** to each tube.